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Award Number: DAMD17-01-1-0167

TITLE: Are diadenosine polyphosphates and/or FHIT involved in anoikis?

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REPORT DATE: June 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 2002	Annual (1 Jun 01 - 31 May 02)

4. TITLE AND SUBTITLE
Are diadenosine polyphosphates and/or FHIT involved in anoikis?

5. FUNDING NUMBERS
DAMD17-01-1-0167

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

Anoikis protects the organism against the inappropriate growth of epithelial cells released during normal turnover. Downstream of the "anoikis receptors", many signaling components have been identified, but many others are undoubtedly remaining to be discovered. One of the primary limitations appears to be the tendency to focus on two second messengers, phosphorylation and lipids. While these are unquestionably important, many other signaling mediators are emerging at present, whose role in anoikis is totally unexplored, even though they (and others) may potentially be essential to the mechanism.

In this light, we propose to examine the role of a class of molecules represented by diadenosine triphosphate (Ap3A) and diadenosine tetraphosphate (Ap4A) in anoikis. These molecules occur in all organisms, accumulate in response to cellular stress, and have quite recently been implicated in apoptosis in mammalian cells.

A tumor suppressor gene that is frequently altered in various human cancers, FHIT (Fragile Histidine Triad), is an Ap3A and Ap4A hydrolase, connecting these dinucleotides with cancer. ApnAs probably act as cofactors for Fhit's effector function (analogous to the function of GTP for ras.) The FHIT gene is altered in 82% of BRCA2-linked breast carcinomas and 40% of sporadic cases, implicating FHIT as a breast cancer-relevant, ApnA-regulated protein that may be involved in regulating apoptosis.

Given the particular importance of anoikis-resistance in the development of breast cancer, the purpose of this IDEA project is to determine whether Ap3A/Ap4A and/or FHIT can regulate anoikis in normal and transformed mammary epithelial cells; the second goal is to establish the functional relationship between mammary tumor-related oncogenes and this new component in regulating anoikis.

14. SUBJECT TERMS	15. NUMBER OF PAGES
breast cancer, anoikis, ApnA, Fhit, extracellular matrix, apoptosis	5
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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INTRODUCTION AND BODY

The FHIT (Fragile Histidine Triad) protein is an intriguing tumor suppressor protein. The FHIT gene is altered in a large fraction of both sporadic and familial human breast cancers. Yet, the biochemical activity and biologic function of FHIT protein are unknown at present.
This project seeks to address the possible function of FHIT protein in anoikis. Anoikis is the apoptotic response to cell-matrix detachment; anoikis prevents mammary epithelial cells from colonizing in novel locations (i.e., metastasizing.)

KEY RESEARCH ACCOMPLISHMENTS

Our work so far has focussed on Task #2 (To test the ability of FHIT to regulate anoikis in mammary epithelial cells) and Task #4 (To test whether FHIT is a core component of the apoptotic machinery.) In addition, we have adopted a new approach with regard to the chromosomal stability of the FHIT gene that is discussed below.

- Task #2: Subsequent to the submission of this grant application, we learned of the short-interfering RNA (siRNA) approach to protein ablation and have begun to design a FHIT-ablation system using this technology to analyze the role of FHIT in anoikis. We have both designed siRNAs for FHIT (synthesized by Dharmacon, Inc.) and designed double –stranded oligonucleotides coding for FHIT siRNA that have been subcloned into the pSuppress vectors described by (Paul et al., Nat. Biotechnol. 20:505-508) that permits stable expression of the siRNA in transfected cells. We are working primarily with the MCF10a cell line because it is an anoikis-sensitive normal mammary epithelial cell line in which other siRNAs have been used effectively. Preliminary experiments addressing testing the reduction of FHIT levels on anoikis are in progress.

- Task #4. In order to address the role of FHIT in apoptosis, we have decided to focus on the yeast two-hybrid approach to identify FHIT-interacting proteins. In this connection, we have subcloned FHIT into the yeast lexA-bait plasmid pGilda and verified that FHIT neither activates nor represses transcription in the yeast two hybrid strain EGY48. We are currently screening an MCF7 cell-derived library for FHIT-interacting clones.

- New Task: It has recently been shown that mutations in genome surveillance factors such as BRCA1 or MLH1 – or other components of the BASC (BRCA1-associated genome surveillance complex) –control the stability of the FHIT coding sequence (see Turner et al., Cancer Res. 62:4054-4060, 2002). Interestingly, we have recently found (R. Screaton and S. Frisch, manuscript in preparation) that FAS-associated death domain protein (FADD) is a nuclear protein that is a component of the BASC complex : it interacts with MLH1 indirectly through a bridge protein called MBD4 (Methyl-CG Binding Domain 4.) We hypothesize that MLH1 is a DNA damage sensor that triggers apoptosis through FADD under certain conditions. Thus, the prediction is that cells lacking FADD have microsatellite instability, which would

lead to FHIT mutations. This hypothesis is currently being explored by the use of FADD-knockout cells, using PCR-based assays for FHIT and other gene targets to screen for hypermutability.

REPORTABLE OUTCOMES: N/A

CONCLUSIONS:

1. SiRNA-mediated ablation of FHIT may provide a feasible approach for testing the role of FHIT in anoikis; 2. FHIT protein may interact with known apoptosis regulatory proteins, establishing a mechanistic foothold for understanding the function of FHIT; 3. The stability of the FHIT/FRA3 locus may be partly controlled by FADD protein.

REFERENCES:

1. Paul, C.P., Good, P.D., Winer, I. & Engelke, D.R. Effective expression of small interfering RNA in human cells. Nat. Biotechnol. 20:505-508, 2002.

2. Turner, B.C., Ottey, M., Zimonjic, D.B., Potoczek, M., Hauck, W.W., Pequignot, E., Keck-Waggoner, C.L., Sevignani, C., Aldaz, C.M., McCue, P.A., Palazzo, J., Huebner, K. & Popescu, N.C. The *Fragile Histidine Triad/Common Chromosome Fragile Site 3B* Locus and Repair-deficient Cancers. Cancer Res. 62:4054-4060, 2002.

APPENDICES: N/A